Amended Pages to Specification

[00126] An antibody heavy chain can be modified using oligonucleotide mutagenesis. Briefly, the desired DNA is altered by hybridizing an oligonucleotide encoding a mutation to a DNA template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the desired protein. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected alteration in the desired protein DNA. Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al. (*Proc. Natl. Acad. Sci.* USA, 75: 5765[1978]).

[00127] To effectuate a change from serine to proline at amino acid number 241 of the hinge region, oligonucleotide mutagenesis can be employed using the oligo S241P that will change the serine to proline. The resulting mutant form can be used to generate transgenic mice. The transgenic mice can be milked, and the milk tested for the presence of the antibody and the relative amount of the "half molecule." The sequence of a hinge region of an IgG4 antibody and the oligonucleotideS241P which can be used to mutagenize it are as follows:

IGG4 HINGE REGION

1668 TCTGCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA

GGTAAGCCAACCCAGGCCT (SEQ ID NO. 1)

$^{R/S}$ Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro (SEQ ID NO. 2)

S241P OLIGO

GGT CCC CCA TGT CCT CCC TGC CCA GGT AAG CCA (SEQ ID NO. 3)

$^{R/S}$ Gly Pro Pro Cys Pro Pro Cys Pro Gly Lys Pro (SEQ ID NO. 4)

[00128] Further, the entire hinge region of an IgG antibody can be replaced with the hinge region of another antibody. To effectuate this change, an oligonucleotide that codes for the an exon containing the replacement hinge region can be used. The sequence of a hinge

region of an IgG4 antibody and an oligonucleotide which contains an IgG2 replacement hinge region are as follows:

IGG4 HINGE REGION

1662 CTTCTCTCTGCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA GGTCCGCCAACCCAGGC (SEQ ID NO. 5)

R/S Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro(SEQ ID NO. 6)

IGG2 HINGE REGION

1729 CTTCTCTCTGCA GAG CGC AAA TGT TGT GTC GAG TGC CCA CCG TGC CCA GGTCCGCCAACCCAGGC (SEQ ID NO. 7)

R/S Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro (SEQ ID NO. 8)

[00129] The N-linked glycosylation site on the CH2 of an IgG heavy chain can be eliminated via oligonucleotide mutagenesis using an oligo that causes a change from asparagine to glutamine in the consensus site. The sequence of an oligonucleotide that can effectuate such a change is as follows:

2014 GAG GAG CAG TTC CAG TCT ACT TAC CGA GTG GTC (SEQ ID NO. 9)

R/S Glu Glu Gln Phe Gln Ser Thr Tyr Arg Val Val (SEQ ID NO. 10)

Testing of Mutagenized Versions of Antibodies

[00130] The light chain and mutagenized heavy chain are ligated to the casein promoter and used to generate transgenic mice. Mice are then tested for expression of the antibody as well as the half antibody.

Transgenic Animals

[00131] A founder (F_0) transgenic goat can be made by transfer of fertilized goat eggs that have been microinjected with a construct. The methodologies that follow in this section can be used to generate transgenic goats. The skilled practitioner will appreciate that such procedures can be modified for use with other animals.

Goat Species and Breeds: